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(54) Title: ANTAGONIST ANTI-CD40 MONOCLONAL ANTIBODY THERAPY FOR MULTIPLE SCLEROSIS TREATMENT

(57) Abstract: The present invention is directed to a method for preventing or alleviating the symptons of MS by delivering to the central nervous system an interferon and an anti-CD40 antibody. Interferons include interferon-β and its muteins such as IFN-β_{ser17}.

ANTAGONIST ANTI-CD40 MONOCLONAL ANTIBODY THERAPY FOR MULTIPLE SCLEROSIS TREATMENT

TECHNICAL FIELD

The invention relates to treatment of B and T cell mediated autoimmune disease in humans using antibodies to CD40 alone or in combination with an immunomodulating agent such as interferon-β-1b.

BACKGROUND OF THE INVENTION

Multiple sclerosis (MS) is one of several diseases believed to result from an immunoregulatory defect. It results in a chronic, disabling condition of the central nervous system, and symptoms can range from mild numbness to paralysis and loss of vision. Most patients are diagnosed between the ages of 20 and 40, and therefore face a lifetime of symptoms and treatment. The progress of the disease cannot be predicted, and the treatment in one patient may not be predictive for treatment of another patient.

Histologically, the symptoms are associated with destruction of myelin, the fatty sheath surrounding nerve fibers. As a result of the damage, the nerve impulses are slowed or stopped, producing physical symptoms such as muscle weakness, tremor, vision problems, lack of balance, pain, and fatigue, and psychological changes including mood swings, forgetfulness, and difficulty in concentrating. Although existing therapies can alleviate some or all of the symptoms in a given patient, the therapy must be carefully monitored and can be contra-indicated if the symptoms worsen or the patient experiences a relapse.

At present, 250,000 to 350,000 people in the U.S. are believed to be living with MS. Because it strikes during a person's most active years, it takes a huge toll on the quality of life and productivity of both the patient and the family. Although the early onset of symptoms is the most common pattern, almost 10% of patients experience the first symptoms after age 50. In this age group, diagnosis of often

complicated by the presence of other conditions, leading to some degree of misdiagnosis.

The most common treatment for MS at the present time is β-interferon (IFN-β), which exerts its effect through modulation of the immune system. Specifically, β-interferon binds to receptors of immune system cells. However, β-interferon only temporarily slows progression of disease in a fraction of patients.

There is a need in the art for therapeutic compositions such as anti-CD40 antibodies that, alone or in combination with interferon, slow the progression of disease in MS patients.

SUMMARY OF THE INVENTION

It is an object of this invention to provide a method for preventing or treating an autoimmune disease in a patient, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a human monoclonal antibody capable of binding to a human CD40 antigen located on the surface of a CD40-bearing cell such as a human B cell or other antigen presenting cell, wherein the binding of the antibody to the CD40 antigen prevents the priming of pathogenic T-cells, alone or in combination with or co-administered with interferon in a pharmaceutically acceptable excipient.

It is another object of this invention to provide a method for preventing or treating multiple sclerosis in a patient, the method comprising administering to a patient in need of such treatment a therapeutically effective amount of a human monoclonal antibody capable of binding to a human CD40 antigen located on the surface of a human antigen presenting cell, wherein the binding of the antibody to the CD40 antigen prevents the priming of pathogenic T-cells, alone or in combination with or co-administered with interferon. It is a further object of the invention to provide a composition for administration to a patient in order to prevent or treat a B cell mediated disease, wherein the composition comprises an anti-CD40 antibody and a type 1 interferon, in a pharmaceutically acceptable excipient.

In more preferred embodiments of the above objects, the monoclonal antibody is 15B8, 20C4, 13E4, 12D9, or 9F7. The monoclonal antibody, 15B8, is a human anti-CD40 monoclonal antibody.

In other preferred embodiments of the above objects, the interferon is a type 1 interferon. In other preferred embodiments, the type 1 interferon is interferon-α, interferon-β, interferon-δ, interferon-ω (interferon-α II1) or interferon-τ (trophoblast interferon). In other preferred embodiments of the above objects, the interferon is interferon-β-1b. In other preferred embodiments of the above objects, the interferon is interferon-β-1a.

In specific embodiments of the invention, the interferon and antibody compositions are administered to a tissue of the mammal innervated by the trigeminal nerve, the olfactory nerve, or a combination thereof, wherein the interferon is absorbed through the tissue and transported to the central nervous system of the mammal. In other preferred embodiments of the above objects the interferon and antibody compositions are administered to a tissue of the mammal by intravenous injection. In other preferred embodiments of the above objects the interferon and antibody compositions are administered to a tissue of the mammal by subcutaneous or intramuscular injection.

The invention provides a method for preventing or alleviating a B cell or other antigen presenting cell mediated disease of the central nervous system in a mammal wherein said disease is responsive to treatment with interferon, comprising: administering a composition comprising an interferon to a tissue of the mammal innervated by the trigeminal nerve, the olfactory nerve, or a combination thereof, wherein the interferon is absorbed through the tissue and transported to the central nervous system of the mammal; and administering a composition comprising an anti-CD40 antibody to said mammal.

In certain embodiments of the method, the tissue comprises a nasal cavity tissue, a conjunctiva, an oral tissue, or a skin. In other embodiments of the method, administering the interferon to the conjunctiva comprises administering the interferon between a lower eyelid and an eye.

In further embodiments of the method, administering the interferon to the skin comprises administering the interferon to a face, a forehead, an upper eyelid, a lower eyelid, a dorsum of the nose, a side of the nose, an upper lip, a cheek, a chin, a scalp, or a combination thereof.

In a specific embodiment of the method, administering the interferon to the oral tissue comprises sublingual administration.

In other embodiments of the method, the interferon is selected from the group consisting of interferon-alpha (IFN-α), interferon-beta (IFN-β), and biologically active variants thereof, and the anti-CD40 antibody is a monoclonal antibody.

In specific embodiments, the interferon is native human IFN-β or a biologically active variant thereof. In other specific embodiments, the IFN-β variant comprises an amino acid sequence having at least 70% sequence identity to native human IFN-β. In yet a further specific embodiment, the interferon is administered to an upper one third of a nasal cavity.

In other embodiments of the method, the interferon is transported to a cerebellum, a superior colliculus, a periventricular white matter, an optic nerve, a midbrain, a pons, an olfactory bulb, an anterior olfactory nucleus, or any combination thereof, or to a spinal cord, a brain stem, a cortical structure, a subcortical structure, or any combination thereof.

In certain embodiments, the interferon is administered in a dosage range of about 0.14 nmol/kg of brain weight to about 138 nmol/kg of brain weight. In specific embodiments, the interferon is native human IFN-β or a biologically active variant thereof.

The invention provides a method of preventing or alleviating multiple sclerosis.

The invention further provides a method of prolonging the effectiveness of interferon treatment of multiple sclerosis in a human, comprising administering to the human a composition comprising an anti-CD40 antibody. In certain embodiments of this method, the antibody is selected from the group consisting of 15B8, 20C4, 13E4, 12D9, 9F7 and 5D12.

DETAILED DESCRIPTION OF THE INVENTION

The CD40 antigen is a glycoprotein expressed on the cell surface of B cells and other antigen-presenting cells, including dendritic cells. During B-cell differentiation, the molecule is first expressed on pre-B cells and then disappears from the cell surface when the B cell becomes a plasma cell. Crosslinking of the CD40

molecules with anti-CD40 antibodies mediates a variety of effects on B cells. The CD40 antigen is known to be related to the human nerve growth factor (NGF) receptor and tumor necrosis factor-α (TNF-α) receptor, suggesting that CD40 is a receptor for a ligand with important functions in B-cell activation.

CD40 is a key element of immune responses. Engagement of CD40 on antigen-presenting cells by its ligand, termed CD40L or CD154, causes production of cytokines and up-regulation of co-stimulatory molecules leading to efficient activation of T lymphocytes. Engagement of CD40 on B lymphocytes provides a co-stimulatory signal to the B cell that drives antibody production. Thus, blocking of CD40 engagement and subsequent T-cell activation has the potential to suppress antibody and cell mediated immune responses.

CD40L is a member of the TNF family. It is expressed on activated T cells and B cells, and on endothelial cells, mast cells, eosinophils and basophils. CD40L is expressed as both membrane-bound and soluble-secreted forms. Several functions of CD40 and CD40L interaction have been identified. In B cells, the interaction modulates clonal expansion, Ig production, germinal center formation, isotype switching, induction and maintenance of memory, and affinity maturation. In T cells, the interaction modulates activation of T helper cells and cytotoxic T lymphocytes (CTLs). In macrophages, the interaction modulates stimulation of co-stimulatory functions,. induction of cytokine production, co-stimulation of nitrous oxide (NO) generation, induction of metalloproteinase production and rescue from apoptosis. In dendritic cells, the interaction modulates maturation, induction of cytokine production, antigen-presenting and co-stimulatory function.

The role of CD40-CD40L interaction has been partly elucidated by studying functional defects of CD40L-deficient humans and mice, and CD40-deficient mice. Several effects are seen, including increased susceptibility to intracellular parasites; reduced production of IFNγ and IL-12; impaired T cell priming to peptide antigens; reduced follicular dendritic cell network; absence of germinal center and impaired Ig isotype switching to T-cell dependent antigens. However, there is normal antibody response to T-cell independent antigens. Overall, the effects are mediated through the impaired immune system. No defect has been identified in other systems. An anti-

HuCD40 blocking antibody has been developed, the mouse monoclonal antibody (5D12). The antibody inhibits *in vitro* MLR and B cell activation and Ig production.

Several effects of 5D12 have been studied *in vitro*. First, 5D12 causes inhibition of IgM secretion by human peripheral blood B cells stimulated with syngeneic human activated T cells. The antibody also causes inhibition of human peripheral B cell proliferation stimulated by anti-CD3 activated-Jurkat cells or CHO-CD40L cells. Inhibition of cytokine secretions stimulated through CD40 in DCs or monocytes was also observed using 5D12. Earlier studies provided preliminary evidence of a role of CD40-CD40L interaction in experimental allergic encephalomyelitis (EAE) and multiple sclerosis. Gerritse et al., (*Proc. Natl. Acad. Sci., 93*:2499-2504, 1996) showed that T-cells expressing CD40L were present in MS brain sections. CD40L+ cells co-localized with CD40-bearing cells (macrophages or monocytes) in active lesions (perivascular infiltrates). Anti-CD40L antibody has been shown to prevent or reduce EAE in mice. Laman (*J. Neuroimmunology 86*:30-45, 1998) observed abundant expression of CD40 in perivascular infiltrates of macrophages in marmoset monkeys with acute EAE.

The present invention relates to a method of treating multiple sclerosis using anti-CD40 antibodies alone or in combination with a type 1 interferon, for example, interferon-β. Patients with continuing worsening of their disease or significant side effects frequently discontinue therapy with interferon-β. According to the invention, a CD40/CD40L blockade may have synergistic anti-inflammatory or immunomodulatory effects with interferon-β, and may reduce the incidence of auto-antibodies (anti-thyroid, anti-hepatic, etc.). Therefore, co-administration of an anti-CD40 antibody such as 15B8 with an interferon-β, such as interferon-β-1b, may reduce the rate of interferon-β-1b discontinuations.

One target patient population includes all patients currently undergoing treatment with interferon-β-1b (for treatment of any form of MS); patients who have experienced at least one relapse in the previous six months on interferon-β-1b; patients with at least one enhancing lesion at baseline MRI scan; and patients with disease progression in the past six months.

A second target patient population includes all patients newly diagnosed with MS or with clinically isolated syndromes suggestive of MS. These patients will be

treated with anti-CD40 antibody, such as 15B8, co-administered with interferon-β-1b. According to the invention, a CD40/CD40L blockade may have synergistic anti-inflammatory or immunomodulatory effects with interferon-β. Co-administration of anti-CD40 antibody with interferon-β-1b may prolong time to establishment of definite MS or result in increased efficacy for treatment of MS compared to administration of interferon-β-1b alone.

Another target patient population includes all patients diagnosed with rheumatoid arthritis or other autoimmune disease. These patients will be treated with anti-CD40 antibody, such as 15B8, alone or co-administered with interferon-β-1b.

Another target patient population includes all patients diagnosed with central nervous system (CNS) tumors or gliomas, for example, astrocytomas, ependymomas, oligodendrogliomas, and tumors with mixtures of two or more of these cell types. These patients will be treated with anti-CD40 antibody, such as 15B8, co-administered with interferon-β-1b.

Another target patient population includes all patients diagnosed with Crohn's disease, Crohn's colitis or chronic ulcerative colitis. These patients will be treated with anti-CD40 antibody, such as 15B8, alone or co-administered with interferon-β-1b.

Another target patient population includes all patients diagnosed with idiopathic pulmonary fibrosis, which may occur as a result of viral infection or autoimmune disease. These patients will be treated with anti-CD40 antibody, such as 15B8, alone or co-administered with interferon-β-1b.

Other target populations include those undergoing treatment with other interferons. Interferons (IFNs) are a family of molecules encompassing over 20 different proteins and are members of the cytokine family that induce antiviral, antiproliferative, antitumor, and/or cytokine effects. IFNs are relatively small, species-specific, single chain polypeptides, which are produced in response to a variety of inducers, such as mitogens, polypeptides, viruses, and the like. In humans, IFNs are produced, for example, as type I interferon (-α, -β, -ω, or -τ) or type 2 interferon (-γ). Synthetic interferons are also known in the art. *See*, for example, U.S. Patent No. 6,114,145, herein incorporated by reference. According to the '145 patent, a synthetic interferon polypeptide has the amino acid sequence of SEQ ID NO:2 and

in one embodiment is encoded by a polynucleotide of SEQ ID NO:1. Upon secretion from mammalian cells, interferon molecules bind to a receptor on the surface of a target cell and elicit a chain of events, which can alter the amount and activity of protein in the target cell. Such alterations can include, for example, changes in gene transcription or enzymatic activity.

Patients undergoing treatment with biologically active variants of interferon may also be suitable target populations. These variants retain the biological activity of the interferon, for example, IFN-α and IFN-β variants known in the art and as discussed below, and the variants retain the ability to bind their respective receptor sites. Such activity may be measured using standard bioassays. Representative assays detecting the ability of the variant to interact with an interferon receptor type I can be found in, for example, U.S. Patent No. 5,766,864, herein incorporated by reference. Preferably, the variant has at least the same activity as the native molecule.

Suitable biologically active variants can be fragments, analogues, and derivatives of the interferon polypeptides. By "fragment" is intended a protein consisting of only a part of the intact interferon polypeptide sequence. The fragment can be a C-terminal deletion or N-terminal deletion of the interferon polypeptide. By "analogue" is intended either the full length polypeptide or a fragment thereof, wherein the analogue comprises a native polypeptide sequence having one or more amino acid substitutions, insertions, or deletions. Peptides having one or more peptoids (peptide mimics) are also encompassed by the term analogue (*see i.e.,* International Publication No. WO 91/04282). By "derivative" is intended any suitable modification of the native polypeptide or fragments thereof, or their respective analogues, such as glycosylation, phosphorylation, or other addition of foreign moieties, so long as the activity is retained.

Preferably, naturally or non-naturally occurring variants of an interferon have amino acid sequences that are at least 70%, preferably 80%, more preferably, 85%, 90%, 91%, 92%, 93%, 94% or 95% identical to the amino acid sequence to the reference molecule, for example, the native human interferon, or to a shorter portion of the reference interferon molecule. More preferably, the molecules are 96%, 97%, 98% or 99% identical. Percent sequence identity is determined using the Smith-Waterman homology search algorithm using an affine gap search with a gap open

penalty of 12 and a gap extension penalty of 2, BLOSUM matrix of 62. The Smith-Waterman homology search algorithm is taught in Smith and Waterman, *Adv. Appl. Math. 2*:482-489, 1981. A variant may, for example, differ by as few as 1 to 10 amino acid residues, such as 6-10, as few as 5, as few as 4, 3, 2, or even 1 amino acid residue.

With respect to optimal alignment of two amino acid sequences, the contiguous segment of the variant amino acid sequence may have additional amino acid residues or deleted amino acid residues with respect to the reference amino acid sequence. The contiguous segment used for comparison to the reference amino acid sequence will include at least 20 contiguous amino acid residues, and may be 30, 40, 50, or more amino acid residues. Corrections for sequence identity associated with conservative residue substitutions or gaps can be made (*see* Smith-Waterman homology search algorithm).

The art provides substantial guidance regarding the preparation and use of such variants, as discussed further below. A fragment of an interferon polypeptide will generally include at least about 10 contiguous amino acid residues of the full-length molecule, preferably about 15-25 contiguous amino acid residues of the full-length molecule, and most preferably about 20-50 or more contiguous amino acid residues of full-length cytokine polypeptide.

For example, conservative amino acid substitutions may be made at one or more predicted, preferably nonessential amino acid residues. A "nonessential" amino acid residue is a residue that can be altered from the wild-type sequence of an interferon (*i.e.,* IFN-α or IFN-β) without altering its biological activity, whereas an "essential" amino acid residue is required for biological activity. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine), and aromatic side chains

(*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Such substitutions would not be made for conserved amino acid residues, or for amino acid residues residing within a conserved motif.

Alternatively, variant interferon nucleotide sequences can be made by introducing mutations randomly along all or part of a cytokine coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for cytokine biological activity to identify mutants that retain activity. Following mutagenesis, the encoded protein can be expressed by recombinant means in bacteria, yeast, insect cells or mammalian cells.

Alternatively, the interferon can be synthesized chemically, by any of several techniques that are known to those skilled in the peptide art. *See*, for example, Li et al., *Proc. Natl. Acad. Sci. USA* 80:2216-2220, 1983, Steward and Young, *Solid Phase Peptide Synthesis* (Pierce Chemical Company, Rockford, Illinois), 1984, and Baraney and Merrifield, *The Peptides: Analysis, Synthesis, Biology*, ed. Gross and Meinhofer, Vol. 2 (Academic Press, New York, 1980), pp. 3-254, discussing solid-phase peptide synthesis techniques; and Bodansky, *Principles of Peptide Synthesis* (Springer-Verlag, Berlin, 1984) and Gross and Meinhofer, eds., *The Peptides: Analysis, Synthesis, Biology*, Vol. 1 (Academic Press, New York, 1980), discussing classical solution synthesis. The interferon can also be chemically prepared by the method of simultaneous multiple peptide synthesis. *See*, for example, Houghten, *Proc. Natl. Acad. Sci. USA 82*:5131-5135, 1984; and U.S. Patent No. 4,631,211.

The interferon used in the methods of the invention can be from any animal species including, but not limited to, avian, canine, bovine, porcine, equine, and human. Preferably, the interferon is from a mammalian species when the interferon is to be used in treatment of a disorder of the CNS, brain or spinal cord, such as MS, and more preferably is from a mammal of the same species as the mammal undergoing treatment for such a disorder.

Interferon-β

The term "IFN-β" as used herein refers to mature native human β-interferon or any biologically active variants thereof, which are sometimes referred to in the art as IFN-β-like polypeptides, *see*, *e.g.*, U.S. Patent No. 4,462,940. Human native IFN-β or

variants, which may be naturally occurring (*e.g.*, allelic variants that occur at the IFN-β locus) or recombinantly produced, have amino acid sequences that are similar to, or substantially similar to the mature native IFN-β sequence. DNA sequences encoding native human IFN-β are available in the art. *See*, for example, Goeddel et al., *Nucleic Acid Res. 8*:4057, 1980 and Tanigachi et al., *Proc. Japan Acad. Sci. 855*:464, 1979. Fragments of IFN-β or truncated forms of IFN-β that retain their activity are also encompassed. These biologically active fragments or truncated forms of IFN-β are generated by removing amino acid residues from the full-length IFN-β amino acid sequence using recombinant DNA techniques well known in the art. IFN-β polypeptides may be glycosylated or unglycosylated, as both the glycosylated and unglycosylated forms of IFN-β show qualitatively similar specific activities and, therefore, the glycosyl moieties are not involved in and do not contribute to the biological activity of IFN-β.

The IFN-β variants encompassed herein include muteins of the native mature IFN-β sequence, wherein one or more cysteine residues that are not essential to biological activity have been deliberately deleted or replaced with other amino acids (*see, e.g.*, U.S. Patent No. 4,588,585 and EP218825, herein incorporated by reference) to eliminate sites for either intermolecular cross-linking or incorrect intramolecular disulfide bond formation. IFN-β variants of this type include those containing a glycine, valine, alanine, leucine, isoleucine, tyrosine, phenylalanine, histidine, tryptophan, serine, threonine, or methionine substituted for the cysteine found at amino acid 17 of the mature native amino acid sequence. Serine and threonine are the more preferred replacements because of their chemical analogy to cysteine. Serine substitutions are most preferred. Thus, IFN-β variants with one or more mutations that improve, for example, their pharmaceutical utility are also encompassed by the present invention.

Additional changes can be introduced by mutation into the nucleotide sequences encoding IFN-β, thereby leading to changes in the IFN-β amino acid sequence, without altering the biological activity of the interferon. Thus, an isolated nucleic acid molecule encoding an IFN-β variant having a sequence that differs from human IFN-β can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide sequence disclosed herein,

such that one or more amino acid substitutions, additions or deletions are introduced into the encoded IFN-β. Mutations can be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Such IFN-β variants can also be employed in the present invention.

Biologically active IFN-β variants encompassed by the invention also include IFN-β polypeptides that are covalently linked with, for example, polyethylene glycol (PEG) or albumin.

Biologically active variants of IFN-β encompassed by the invention should retain IFN-β activities, particularly the ability to bind to IFN-β receptors or retain immunomodulatory or anti-viral activities. In some embodiments, the IFN-β variant retains at least about 25%, preferably about 50%, and more preferably about 75% or more of the biological activity of the native IFN-β polypeptide. IFN-β variants whose activity is increased in comparison with the activity of the native IFN-β polypeptide are also encompassed. The biological activity of IFN-β variants can be measured by any method known in the art. Examples of such assays can be found in Fellous et al., *Proc. Natl. Acad. Sci USA 79*:3082-3086, 1982; Czerniecki et al., *J. Virol. 49(2)*:490-496, 1984; Mark et al., *Proc. Natl Acad. Sci. USA 81*:5662-5666, 1984; Branca et al., *Nature* 277:221-223, 1981; Williams et al., *Nature 282*:582-586, 1979; Herberman et al., *Nature 277*:221-223, 1979; and Anderson et al., *J. Biol. Chem. 257(19)*:11301-11304, 1982.

Non-limiting examples of IFN-β polypeptides and IFN-β variant polypeptides encompassed by the invention are set forth in Nagata et al., *Nature 284*:316-320, 1980; Goeddel et al., *Nature 287*:411-416, 1980; Yelverton et al., *Nucleic Acids Res. 9*:731-741, 1981; Streuli et al., *Proc. Natl. Acad. Sci. U.S.A. 78*:2848-2852, 1981; EP028033B1, and EP109748B1. *See also* U.S. Patent Nos. 4,518,584; 4,569,908; 4,588,585; 4,738,844; 4,753,795; 4,769,233; 4,793,995; 4,914,033; 4,959,314; 5,545,723; and 5,814,485. These disclosures are herein incorporated by reference. These citations also provide guidance regarding residues and regions of the IFN-β polypeptide that can be altered without the loss of biological activity.

In one embodiment of the present invention, the IFN-β used in the methods of the invention is the mature native human IFN-β polypeptide. In another embodiment, the IFN-β is the mature IFN-β C17S polypeptide. However, the present invention

encompasses other embodiments where the IFN-β is any biologically active IFN-β polypeptide or variant as described elsewhere herein.

In some embodiments of the present invention, the IFN-β is recombinantly produced. By "recombinantly produced IFN-β" is intended IFN-β that has comparable biological activity to native IFN-β and that has been prepared by recombinant DNA techniques. IFN-β can be produced by culturing a host cell transformed with an expression vector comprising a nucleotide sequence that encodes an IFN-β polypeptide. The host cell is one that can transcribe the nucleotide sequence and produce the desired protein, and can be prokaryotic (for example, *E. coli*) or eukaryotic (for example a yeast, insect, or mammalian cell). Examples of recombinant production of IFN-β are given in Mantei et al., *Nature 297*:128, 1982; Ohno et al., *Nucleic Acids Res. 10*:967, 1982; Smith et al., *Mol. Cell. Biol. 3*:2156, 1983, and U.S. Patent Nos. 4,462,940, 5,702,699, and 5,814,485; herein incorporated by reference.

Interferon-α

The term "IFN-α" as used herein refers to a biologically active human α-interferon or any biologically active variants thereof, which are sometimes referred to in the art as IFN-α-like polypeptides. Human alpha interferons comprise a family of about 30 protein species, encoded by at least 14 different genes and about 16 alleles. Such IFN-α polypeptides include IFN-αa, IFN-αB, IFN-αC, IFN-αD, IFN-αH, IFN-αJ, IFN-αJ1, IFN-αJ2 and IFN-αK. Native human IFN-α or variants, which may be naturally occurring (*e.g.*, allelic variants that occur at the IFN-α locus) or recombinantly produced, have amino acid sequences that are similar to, or substantially similar to the mature native IFN-α sequence. DNA sequences encoding human IFN-α are also available in the art. *See*, for example, Goeddel et al., *Nature 290*:20-26, 1981 (Genbank Accession No. V00551 J00209); Nagata et al., *Nature 284*:3126-320, 1980; Bowden et al., *Gene 27*:87-99, 1984 (Genbank Accession No. NM_000605); and Ohara et al., *FEBS Letters 211*:78-82, 1987; all of which are herein incorporated by reference. Fragments of IFN-α or truncated forms of IFN-α that retain their activity are also encompassed. These biologically active fragments or truncated forms of IFN-α are generated by removing amino acid residues from the full-length IFN-α amino acid

sequence using recombinant DNA techniques well known in the art. IFN-α polypeptides may further be glycosylated or unglycosylated.

Additional changes can be introduced by mutation into the nucleotide sequences encoding IFN-α, thereby leading to changes in the IFN-α amino acid sequence, without altering the biological activity of the interferon. Thus, an isolated nucleic acid molecule encoding an IFN-α variant having a sequence that differs from human IFN-α can be created by introducing one or more nucleotide substitutions, additions, or deletions into the corresponding nucleotide sequence disclosed herein, such that one or more amino acid substitutions, additions or deletions are introduced into the encoded IFN-α. Mutations can be introduced by standard techniques. Such variants of IFN-α, include, for example, IFN-α-2a (Roferon-A™), IFN-α-2b (Intron A™), and IFN-α con-1 (Infergen™). Another variant useful in the methods of the present invention is IFN-α2a, which is disclosed in, for example, EP 43980; Meada et al., *PNAS 77*:7010, 1980; and Levy et al., *PNAS 78:*6186, 1981; all of which are herein incorporated by reference. Further variants of IFN-α can be found, for example, in U.S. Patent No. 5,676,942, herein incorporated by reference. These citations also provide guidance regarding residues and regions of the IFN-α polypeptide that can be altered without the loss of biological activity.

Biologically active IFN-α variants used in the methods of the invention also include IFN-α polypeptides that have covalently linked with, for example, polyethylene glycol (PEG) or albumin. *See*, for example, U.S. Patent No. 5,762,923, herein incorporated by reference.

Biologically active variants of IFN-α used in the methods of the invention should retain IFN-α activities, particularly the ability to bind to IFN-α receptors or retain immunomodulatory, antiviral, or antiproliferative activities. In some embodiments, the IFN-α variant retains at least about 25%, preferably about 50%, and more preferably about 75% or more of the biological activity of the native IFN-α polypeptide. IFN-α variants whose activity is increased in comparison with the activity of the native IFN-α polypeptide are also encompassed. The biological activity of IFN-α variants can be measured by any method known in the art. Examples of such assays are describe above.

In some embodiments of the present invention, the IFN-α is recombinantly produced. By "recombinantly produced IFN-α" is intended IFN-α that has comparable biological activity to native IFN-α and that has been prepared by recombinant DNA techniques. IFN-α can be produced by culturing a host cell transformed with an expression vector comprising a nucleotide sequence that encodes an IFN-α polypeptide. The host cell is one that can transcribe the nucleotide sequence and produce the desired protein, and can be prokaryotic (for example, *E. coli*) or eukaryotic (for example a yeast, insect, or mammalian cell). Details of the cloning of interferon-cDNA and the direct expression thereof, especially in E. coli, have in the meantime been the subject of many publications. Thus, for example, the preparation of recombinant interferons is known. *See*, for example, *Nature 295*:503-508, 1982; *Nature 284*:316-320, 1980; *Nature 290:*20-26, 1981; *Nucleic Acids Res. 8:*4057-4074, 1980, as well as European Patents Nos. 32134, 43980 and 211 148. Further examples of recombinant production of IFN-α-2 are provided in Nagata et al., *Nature 284*:316, 1980 and European Patent 32,134. All of these references are herein incorporated by reference.

Pharmaceutical Composition: Interferon

Increases in the amount of interferon in the CNS, brain, and/or spinal cord to a therapeutically effective level may be obtained via administration of a pharmaceutical composition including a therapeutically effective dose of interferon. By "therapeutically effective dose" is intended a dose of interferon that achieves the desired goal of increasing the amount of interferon in a relevant portion of the CNS, brain, and/or spinal cord to a therapeutically effective level enabling a desired biological activity of the interferon.

The invention employs a composition for delivery of interferon to the CNS, brain, and/or spinal cord upon administration to tissue innervated by the olfactory and/or trigeminal nerves. The composition can include, for example, any pharmaceutically acceptable additive, carrier, or adjuvant that is suitable for administering an interferon to tissue innervated by the olfactory and/or trigeminal nerves. Preferably, the pharmaceutical composition can be employed in diagnosis, prevention, or treatment of a disease, disorder, or injury of the CNS, brain, and/or spinal

cord. Preferably, the composition includes an interferon in combination with a pharmaceutical carrier, additive, and/or adjuvant that can promote the transfer of the interferon within or through tissue innervated by the olfactory and/or trigeminal nerves. Alternatively, the interferon may be combined with substances that may assist in transporting the interferon to sites of nerve cell damage. The composition can include one or several interferons.

The composition typically contains a pharmaceutically acceptable carrier mixed with the interferon and other components in the pharmaceutical composition. By "pharmaceutically acceptable carrier" is intended a carrier that is conventionally used in the art to facilitate the storage, administration, and/or the healing effect of the interferon. A carrier may also reduce any undesirable side effects of the interferon. A suitable carrier should be stable, *i.e.*, incapable of reacting with other ingredients in the formulation. It should not produce significant local or systemic adverse effect in recipients at the dosages and concentrations employed for treatment. Such carriers are generally known in the art.

Suitable carriers for this invention include those conventionally used for large stable macromolecules such as albumin, gelatin, collagen, polysaccharide, monosaccharides, polyvinylpyrrolidone, polylactic acid, polyglycolic acid, polymeric amino acids, fixed oils, ethyl oleate, liposomes, glucose, sucrose, lactose, mannose, dextrose, dextran, cellulose, mannitol, sorbitol, polyethylene glycol (PEG), and the like.

Water, saline, aqueous dextrose, and glycols are preferred liquid carriers, particularly for solutions. The carrier can be selected from various oils, including those of petroleum, animal, vegetable or synthetic origin, for example, peanut oil, soybean oil, mineral oil, sesame oil, and the like. Suitable pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol, and the like. The compositions can be subjected to conventional pharmaceutical expedients, such as sterilization, and can contain conventional pharmaceutical additives, such as preservatives, stabilizing interferons, wetting, or emulsifying agents, salts for adjusting osmotic pressure, buffers, and the like.

A composition formulated for intranasal delivery may optionally comprise an odorant. An odorant agent is combined with the interferon to provide an odoriferous sensation, and/or to indicate inhalation of the intranasal preparation to enhance delivery of the active interferon to the olfactory neuroepithelium. The odoriferous sensation provided by the odorant agent may be pleasant, obnoxious, or otherwise malodorous. The odorant receptor neurons are localized to the olfactory epithelium that, in humans, occupies only a few square centimeters in the upper part of the nasal cavity. The cilia of the olfactory neuronal dendrites that contain the receptors are fairly long (about 30-200 um). A 10-30 μm layer of mucus envelops the cilia that the odorant agent must penetrate to reach the receptors. *See* Snyder et al., *J. Biol. Chem. 263*:13972-13974, 1998. Use of a lipophilic odorant agent having moderate to high affinity for odorant binding protein (OBP) is preferred. OBP has an affinity for small lipophilic molecules found in nasal secretions and may act as a carrier to enhance the transport of a lipophilic odorant substance, interferon to the olfactory receptor neurons. It is also preferred that an odorant agent is capable of associating with lipophilic additives such as liposomes and micelles within the preparation to further enhance delivery of the interferons by means of OBP to the olfactory neuroepithelium. OBP may also bind directly to lipophilic agents to enhance transport of the interferon to olfactory neural receptors.

Suitable odorants having a high affinity for OBP include terpanoids such as cetralva and citronellol, aldehydes such as amyl cinnamaldehyde and hexyl cinnamaldehyde, esters such as octyl isovalerate, jasmines such as C1S-jasmine and jasmal, and musk 89. Other suitable odorant agents include those which may be capable of stimulating odorant-sensitive enzymes such as adenylate cyclase and guanylate cyclase, or which may be capable of modifying ion channels within the olfactory system to enhance absorption of the interferon.

Other acceptable components in the composition include, but are not limited to, pharmaceutically acceptable agents that modify isotonicity, including water, salts, sugars, polyols, amino acids, and buffers such as phosphate, citrate, succinate, acetate, and other organic acids or their salts. Typically, the pharmaceutically acceptable carrier also includes one or more stabilizers, reducing agents, anti-oxidants and/or anti-oxidant chelating agents. The use of buffers, stabilizers, reducing agents, anti-

oxidants and chelating agents in the preparation of protein based compositions, particularly pharmaceutical compositions, is well known in the art. *See* Wang et al., *J. Parent. Drug Assn. 34(6)*:452-462, 1980; Wang et al., *J. Parent. Sci. and Tech. 42*:S4-S26, 1988 (Supplement); Lachman et al., *Drug and Cosmetic Industry 102(1)*:36-38, 40, 1968 and 146-148; Akers, M.J., *J. Parent. Sci. and Tech. 36(5)*:222-228, 1988; and Colowick et al. *Methods in Enzymology,* Vol. XXV, p. 185-188, 1988.

Suitable buffers include acetate, adipate, benzoate, citrate, lactate, maleate, phosphate, tartarate, borate, tri(hydroxymethyl aminomethane), succinate, glycine, histidine, the salts of various amino acids, or the like, or combinations thereof. *See* Wang (1980) *supra* at page 455. Suitable salts and isotonicifiers include sodium chloride, dextrose, mannitol, sucrose, trehalose, or the like. Where the carrier is a liquid, it is preferred that the carrier is hypotonic or isotonic with oral, conjunctival or dermal fluids and have a pH within the range of 4.5-8.5. Where the carrier is in powdered form, it is preferred that the carrier is also within an acceptable non-toxic pH range.

Suitable reducing agents, which maintain the reduction of reduced cysteines, include dithiothreitol (DTT; also known as Cleland's reagent) or dithioerythritol at 0.01% to 0.1% wt/wt; acetylcysteine or cysteine at 0.1% to 0.5% and pH 2-3; and thioglycerol at 0.1% to 0.5% and pH 3.5-7.0; and glutathione. *See* Akers (1988) *supra* at pages 225 to 226. Suitable antioxidants include sodium bisulfite, sodium sulfite, sodium metabisulfite, sodium thiosulfate, sodium formaldehyde sulfoxylate, and ascorbic acid. *See* Akers (1988) *supra* at pages 225. Suitable chelating agents, which chelate trace metals to prevent the trace metal catalyzed oxidation of reduced cysteines, include citrate, tartrate, ethylenediaminetetraacetic acid (EDTA) in its disodium, tetrasodium, and calcium disodium salts, and diethylenetriamine pentaacetic acid (DTPA). *See, e.g.*, Wang (1980) *supra* at pages 457-458 and 460-461, and Akers (1988) *supra* at pages 224-227.

The composition can include one or more preservatives such as phenol, cresol, p-aminobenzoic acid, BDSA, sorbitrate, chlorhexidine, benzalkonium chloride, or the like. Suitable stabilizers include carbohydrates such as trehalose or glycerol. The composition can include a stabilizer such as one or more of microcrystalline cellulose, magnesium stearate, mannitol, sucrose to stabilize, for example, the physical form of

the composition; and one or more of glycine, arginine, hydrolyzed collagen, or protease inhibitors to stabilize, for example, the chemical structure of the composition. Suitable suspending additives include carboxymethyl cellulose, hydroxypropyl methylcellulose, hyaluronic acid, alginate, chondroitin sulfate, dextran, maltodextrin, dextran sulfate, or the like. The composition can include an emulsifier such as polysorbate 20, polysorbate 80, pluronic, triolein, soybean oil, lecithins, squalene and squalanes, sorbitan treioleate, or the like. The composition can include an antimicrobial such as phenylethyl alcohol, phenol, cresol, benzalkonium chloride, phenoxyethanol, chlorhexidine, thimerosol, or the like. Suitable thickeners include natural polysaccharides such as mannans, arabinans, alginate, hyaluronic acid, dextrose, or the like; and synthetic ones like the PEG hydrogels of low molecular weight and aforementioned suspending the interferon.

The composition can include an adjuvant such as cetyl trimethyl ammonium bromide, BDSA, cholate, deoxycholate, polysorbate 20 and 80, fusidic acid, or the like, and in the case of DNA delivery, preferably, a cationic lipid. Suitable sugars include glycerol, threose, glucose, galactose, mannitol, and sorbitol. A suitable protein is human serum albumin.

Preferred compositions include one or more of a solubility enhancing additive, preferably a cyclodextrin; a hydrophilic additive, preferably a monosaccharide or oligosaccharide; an absorption promoting additive, preferably a cholate, a deoxycholate, a fusidic acid, or a chitosan; a cationic surfactant, preferably a cetyl trimethyl ammonium bromide; a viscosity enhancing additive, preferably to promote residence time of the composition at the site of administration, preferably a carboxymethyl cellulose, a maltodextrin, an alginic acid, a hyaluronic acid, or a chondroitin sulfate; or a sustained release matrix, preferably a polyanhydride, a polyorthoester, a hydrogel, a particulate slow release depo system, preferably a polylactide co-glycolide (PLG), a depo foam, a starch microsphere, or a cellulose derived buccal system; a lipid-based carrier, preferably an emulsion, a liposome, a niosome, or a micelle. The composition can include a bilayer destabilizing additive, preferably a phosphatidyl ethanolamine; a fusogenic additive, preferably a cholesterol hemisuccinate.

Other preferred compositions for sublingual administration including, for example, a bioadhesive to retain the interferon sublingually; a spray, paint, or swab applied to the tongue; retaining a slow dissolving pill or lozenge under the tongue; or the like. Other preferred compositions for transdermal administration include a bioadhesive to retain the interferon on or in the skin; a spray, paint, cosmetic, or swab applied to the skin; or the like.

These lists of carriers and additives is by no means complete and a worker skilled in the art can choose excipients from the GRAS (generally regarded as safe) list of chemicals allowed in the pharmaceutical preparations and those that are currently allowed in topical and parenteral formulations.

For the purposes of this invention, the pharmaceutical composition comprising the interferon can be formulated in a unit dosage and in a form such as a solution, suspension, or emulsion. The interferon may be administered to tissue innervated by the trigeminal and/or olfactory neurons as a powder, a granule, a solution, a cream, a spray (*e.g.*, an aerosol), a gel, an ointment, an infusion, an injection, a drop, or sustained-release composition, such as a polymer disk. For buccal administration, the compositions can take the form of tablets or lozenges formulated in a conventional manner. For administration to the eye or other external tissues, *e.g.*, mouth and skin, the compositions can be applied to the infected part of the body of the patient as a topical ointment or cream. The compounds can be presented in an ointment, for instance with a water-soluble ointment base, or in a cream, for instance with an-oil-in water cream base. For conjunctival applications, the interferon can be administered in biodegradable or non-degradable ocular inserts. The drug may be released by matrix erosion or passively through a pore as in ethylene-vinylacetate polymer inserts. For other mucosal administrations, such as sublingual, powder discs may be placed under the tongue.

Other preferred forms of compositions for administration include a suspension of a particulate, such as an emulsion, a liposome, an insert that releases the interferon slowly, and the like. The powder or granular forms of the pharmaceutical composition may be combined with a solution and with a diluting, dispersing, or surface-active interferon. Additional preferred compositions for administration include a bioadhesive to retain the interferon at the site of administration; a spray,

paint, or swab applied to the mucosa or epithelium; a slow dissolving pill or lozenge; or the like. The composition can also be in the form of lyophilized powder, which can be converted into a solution, suspension, or emulsion before administration. The pharmaceutical composition including interferon is preferably sterilized by membrane filtration and is stored in unit-dose or multi-dose containers such as sealed vials or ampoules.

The method for formulating a pharmaceutical composition is generally known in the art. A thorough discussion of formulation and selection of pharmaceutically acceptable carriers, stabilizers, and isomolytes can be found in *Remington's Pharmaceutical Sciences* (18th ed.; Mack Publishing Company, Eaton, Pennsylvania, 1990), herein incorporated by reference.

The interferon can also be formulated in a sustained-release form to prolong the presence of the pharmaceutically active interferon in the treated mammal, generally for longer than one day. Many methods of preparation of a sustained-release formulation are known in the art and are disclosed in *Remington's Pharmaceutical Sciences* (18th ed.; Mack Publishing Company, Eaton, Pennsylvania, 1990), herein incorporated by reference.

Generally, the interferon can be entrapped in semipermeable matrices of solid hydrophobic polymers. The matrices can be shaped into films or microcapsules. Examples of such matrices include, but are not limited to, polyesters, copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., *Biopolymers 22*:547-556, 1983), polylactides (U.S. Patent No. 3,773,919 and EP 58,481), polylactate polyglycolate (PLGA) such as polylactide-co-glycolide (*see*, for example, U.S. Patent Nos. 4,767,628 and 5,654,008), hydrogels (*see*, for example, Langer et al., *J. Biomed. Mater. Res. 15*:167-277, 1981; Langer, *Chem. Tech. 12*:98-105, 1982), non-degradable ethylene-vinyl acetate (*e.g.* ethylene vinyl acetate disks and poly(ethylene-co-vinyl acetate)), degradable lactic acid-glycolic acid copolyers such as the Lupron Depot™, poly-D-(-)-3-hydroxybutyric acid (EP 133,988), hyaluronic acid gels (*see*, for example, U.S. Patent 4,636,524), alginic acid suspensions, and the like.

Suitable microcapsules can also include hydroxymethylcellulose or gelatin-microcapsules and polymethyl methacrylate microcapsules prepared by coacervation techniques or by interfacial polymerization. See the PCT publication WO 99/24061

entitled "*Method for Producing Sustained-release Formulations*," wherein a protein is encapsulated in PLGA microspheres, herein incorporated by reference. In addition, microemulsions or colloidal drug delivery systems such as liposomes and albumin microspheres, may also be used. *See Remington's Pharmaceutical Sciences* (18th ed.; Mack Publishing Company Co., Eaton, Pennsylvania, 1990). Other preferred sustained-release compositions employ a bioadhesive to retain the interferon at the site of administration.

Among the optional substances that may be combined with the interferon in the pharmaceutical composition are lipophilic substances that can enhance absorption of the interferon through the mucosa or epithelium of the nasal cavity, or along a neural, lymphatic, or perivascular pathway to damaged nerve cells in the CNS. The interferon may be mixed with a lipophilic adjuvant alone or in combination with a carrier, or may be combined with one or several types of micelle or liposome substances. Among the preferred lipophilic substances are cationic liposomes included of one or more of the following: phosphatidyl choline, lipofectin, a lipid-peptoid conjugate, a synthetic phospholipid such as phosphatidyl lysine, or the like. These liposomes may include other lipophilic substances such as gangliosides and phosphatidylserine (PS). Also preferred are micellar additives such as GM-1 gangliosides and phosphatidylserine (PS), which may be combined with the interferon either alone or in combination. GM-1 ganglioside can be included at 1-10 mole percent in any liposomal compositions or in higher amounts in micellar structures. Protein interferons can be either encapsulated in particulate structures or incorporated as part of the hydrophobic portion of the structure depending on the hydrophobicity of the active interferon.

One preferred liposomal formulation employs Depofoam. Interferon can be encapsulated in multivesicular liposomes, as disclosed in the WO publication 99/12522 entitled "*High and Low Load Formulations of IGF-I in Multivesicular Liposomes*," herein incorporated by reference. The mean residence time of interferon at the site of administration can be prolonged with a Depofoam composition.

Administering the Interferon

The total amount of interferon administered per dose should be in a range sufficient to delivery a biologically relevant amount of the interferon (*i.e.*, an amount sufficient to produce a therapeutical effect). The pharmaceutical composition having a unit dose of interferon can be in the form of solution, suspension, emulsion, or a sustained-release formulation. The total volume of one dose of the pharmaceutical composition can range from about 10 µl to about 100 µl, for example, for nasal administration. It is apparent that the suitable volume can vary with factors such as the size of the tissue to which the interferon is administered and the solubility of the components in the composition.

It is recognized that the total amount of interferon administered as a unit dose to a particular tissue will depend upon the type of pharmaceutical composition being administered, that is whether the composition is in the form of, for example, a solution, a suspension, an emulsion, or a sustained-release formulation. For example, where the pharmaceutical composition comprising a therapeutically effective amount of interferon is a sustained-release formulation, interferon is administered at a higher concentration. Needle-free subcutaneous administration to an extranasal tissue innervated by the trigeminal nerve may be accomplished by use of a device which employs a supersonic gas jet as a power source to accelerate an agent that is formulated as a powder or a microparticle into the skin. The characteristics of such a delivery method will be determined by the properties of the particle, the formulation of the agent and the gas dynamics of the delivery device. Similarly, the subcutaneous delivery of an aqueous composition can be accomplished in a needle-free manner by employing a gas-spring powered hand held device to produce a high force jet of fluid capable of penetrating the skin. Alternatively, a skin-patch formulated to mediate a sustained release of a composition can be employed for the transdermal delivery of a neuroregulatory agent to a tissue innervated by the trigeminal nerve. Where the pharmaceutical composition comprises a therapeutically effective amount of an agent, or a combination of agents, in a sustained-release formulation, the agent(s) is/are administered at a higher concentration.

It should be apparent to a person skilled in the art that variations may be acceptable with respect to the therapeutically effective dose and frequency of the

administration of an interferon in this embodiment of the invention. The amount of the interferon administered will be inversely correlated with the frequency of administration. Hence, an increase in the concentration of interferon in a single administered dose, or an increase in the mean residence time in the case of a sustained-release form of interferon, generally will be coupled with a decrease in the frequency of administration.

In the practice of the present invention, additional factors should be taken into consideration when determining the therapeutically effective dose of interferon and frequency of its administration. Such factors include, for example, the size of the tissue, the area of the surface of the tissue, the severity of the disease or disorder, and the age, height, weight, health, and physical condition of the individual to be treated. Generally, a higher dosage is preferred if the tissue is larger or the disease or disorder is more severe.

Some minor degree of experimentation may be required to determine the most effective dose and frequency of dose administration, this being well within the capability of one skilled in the art once apprised of the present disclosure.

For the treatment of a disorder of the CNS in a human, including neurologic, viral, proliferative or immunomodulatory disorders, a therapeutically effective amount or dose of interferon is about 0.14 nmol/kg of brain weight to about 138 nmol/kg brain weight and about 0.14 nmol/kg of brain weight to about 69 nmol/kg of brain weight. In some regimens, therapeutically effective doses for administration of interferon include about 0.13-140 nmol/kg of brain weight. For the treatment of a disorder of the CNS in a human, including neurologic, viral, proliferative or immunomodulatory disorders, the therapeutically effective amount or dose of IFN-β or biologically active variant thereof is about 0.14 nmol/kg of brain weight to about 138 nmol/kg of brain weight and about 0.14 nmol/kg of brain weight to about 69 nmol/kg of brain weight. In some regimens, therapeutically effective doses for administration of IFN-β include about 0.13-140 nmol/kg of brain weight.

It is further recognized that the therapeutically effective amount or dose of interferon to a human may be lower when the interferon is administered via the nasal lymphatics to various tissues of the head and neck for the treatment or prevention of disorders or diseases characterized by immune and inflammatory responses (*i.e.*,

diseases resulting in acute or chronic inflammation and/or infiltration by lymphocytes). In these embodiments, while the interferon can be administered in the dosage range provided above, the interferon may also be administered from about 0.02 to about 138 pmol/kg of brain weight. Alternatively, the interferon may be administered from about 0.02-140 pmol/kg of brain weight. Similarly, when the interferon is IFN-β, the dosage range may also be from about 0.02 to about 140 pmol/kg of brain weight. Alternatively, the interferon may be administered from about 0.02-140 pmol/kg of brain weight.

These doses depend on factors including the efficiency with which the interferon is transported to the CNS or lymphatic system. A larger total dose can be delivered by multiple administrations of the agent.

Intermittent Dosing

In another embodiment of the invention, the pharmaceutical composition comprising the therapeutically effective dose of interferon is administered intermittently. By "intermittent administration" is intended administration of a therapeutically effective dose of interferon, followed by a time period of discontinuance, which is then followed by another administration of a therapeutically effective dose, and so forth. Administration of the therapeutically effective dose may be achieved in a continuous manner, as for example with a sustained-release formulation, or it may be achieved according to a desired daily dosage regimen, as for example with one, two, three or more administrations per day. By "time period of discontinuance" is intended a discontinuing of the continuous sustained-released or daily administration of interferon. The time period of discontinuance may be longer or shorter than the period of continuous sustained-release or daily administration. During the time period of discontinuance, the interferon level in the relevant tissue is substantially below the maximum level obtained during the treatment. The preferred length of the discontinuance period depends on the concentration of the effective dose and the form of interferon used. The discontinuance period can be at least 2 days, preferably is at least 4 days, more preferably is at least 1 week and generally does not exceed a period of 4 weeks. When a sustained-release formulation is used, the discontinuance period must be extended to account for the greater residence time of interferon at the site of injury. Alternatively, the frequency of administration of the

effective dose of the sustained-release formulation can be decreased accordingly. An intermittent schedule of administration of interferon can continue until the desired therapeutic effect, and ultimately treatment of the disease or disorder, is achieved.

In yet another embodiment, intermittent administration of the therapeutically effective dose of interferon is cyclic. By "cyclic" is intended intermittent administration accompanied by breaks in the administration, with cycles ranging from about 1 month to about 2-6 months. For example, the administration schedule might be intermittent administration of the effective dose of interferon, wherein a single short-term dose is given once per week for 4 weeks, followed by a break in intermittent administration for a period of 3 months, followed by intermittent administration by administration of a single short-term dose given once per week for 4 weeks, followed by a break in intermittent administration for a period of 2 months, and so forth. As another example, a single short-term dose may be given once per week for 2 weeks, followed by a break in intermittent administration for a period of 1 month, followed by a single short-term dose given once per week for 2 weeks, followed by a break in intermittent administration for a period of 1 month, and so forth. A cyclic intermittent schedule of administration of interferon to subject may continue until the desired therapeutic effect, and ultimately treatment of the disorder or disease, is achieved.

Neuronal Transport

One embodiment of the present method includes administration of the interferon to the subject in a manner such that the interferon is transported to the lymphatic system, the lacrimal gland, CNS, brain, and/or spinal cord along a neural pathway. A neural pathway includes transport within or along a neuron, through or by way of lymphatics running with a neuron, through or by way of a perivascular space of a blood vessel running with a neuron or neural pathway, through or by way of an adventitia of a blood vessel running with a neuron or neural pathway, or through an hemangiolymphatic system. The invention prefers transport of interferon by way of a neural pathway, rather than through the circulatory system, so that interferon that are unable to, or only poorly, cross the blood-brain barrier from the bloodstream into the brain can be delivered to the lymphatic system, CNS, brain, and/or spinal cord. The

interferon, once past the blood-brain barrier and in the CNS, can then be delivered to various areas of the brain or spinal cord through lymphatic channels, through a perivascular space, or transport through or along neurons. In one embodiment, the interferon preferably accumulates in areas having the greatest density of receptor or binding sites for the interferon.

Use of a neural pathway to transport interferon to the lymphatic system, lacrimal gland, brain, spinal cord, or other components of the central nervous system obviates the obstacle presented by the blood-brain barrier so that medications that cannot normally cross that barrier, can be delivered directly to the brain, cerebellum, brain stem, or spinal cord. Although the interferon that is administered may be absorbed into the bloodstream as well as the neural pathway, the interferon preferably provides minimal effects systemically. In addition, the invention can provide for delivery of a more concentrated level of the interferon to neural cells since the interferon does not become diluted in fluids present in the bloodstream. As such, the invention provides an improved method for delivering interferon to the lymphatic system, CNS, brain, and/or spinal cord.

The Olfactory Neural Pathway

One embodiment of the present method includes delivery of the interferon to the subject in a manner such that the interferon is transported into the CNS, brain, and/or spinal cord along an olfactory neural pathway. Typically, such an embodiment includes administering the interferon to tissue innervated by the olfactory nerve and inside the nasal cavity. The olfactory neural pathway innervates primarily the olfactory epithelium in the upper third of the nasal cavity, as described above. Application of the interferon to a tissue innervated by the olfactory nerve can deliver the interferon to damaged neurons or cells of the CNS, brain, and/or spinal cord. Olfactory neurons innervate this tissue and can provide a direct connection to the CNS, brain, and/or spinal cord due, it is believed, to their role in olfaction.

Delivery through the olfactory neural pathway can employ lymphatics that travel with the olfactory nerve to the various brain areas and from there into dural lymphatics associated with portions of the CNS, such as the spinal cord. Transport along the olfactory nerve can also deliver interferon to an olfactory bulb. A

perivascular pathway and/or a hemangiolymphatic pathway, such as lymphatic channels running within the adventitia of cerebral blood vessels, can provide an additional mechanism for transport of therapeutic interferon to the brain and spinal cord from tissue innervated by the olfactory nerve.

Interferon can be administered to the olfactory nerve, for example, through the olfactory epithelium. Such administration can employ extracellular or intracellular (*e.g.*, transneuronal) anterograde and retrograde transport of the interferon entering through the olfactory nerves to the brain and its meninges, to the brain stem, or to the spinal cord. Once the interferon is dispensed into or onto tissue innervated by the olfactory nerve, the interferon may transport through the tissue and travel along olfactory neurons into areas of the CNS including the brain stem, cerebellum, spinal cord, olfactory bulb, and cortical and subcortical structures.

Delivery through the olfactory neural pathway can employ movement of interferon into or across mucosa or epithelium into the olfactory nerve or into a lymphatic, a blood vessel perivascular space, a blood vessel adventitia, or a blood vessel lymphatic that travels with the olfactory nerve to the brain and from there into meningial lymphatics associated with portions of the CNS such as the spinal cord. Blood vessel lymphatics include lymphatic channels that are around the blood vessels on the outside of the blood vessels. This also is referred to as the hemangiolymphatic system. Introduction of interferon into the blood vessel lymphatics does not necessarily introduce the interferon into the blood.

The Trigeminal Neural Pathway

One embodiment of the present method includes delivery of the interferon to the subject in a manner such that the interferon is transported into the CNS, brain, and/or spinal cord along a trigeminal neural pathway. Typically, such an embodiment includes administering the interferon to tissue innervated by the trigeminal nerve including inside and outside the nasal cavity. The trigeminal neural pathway innervates various tissues of the head and face, as described above. In particular, the trigeminal nerve innervates the nasal, sinusoidal, oral and conjunctival mucosa or epithelium, and the skin of the face. Application of the interferon to a tissue innervated by the trigeminal nerve can deliver the interferon to damaged neurons or

cells of the CNS, brain, and/or spinal cord to cells of the lymphatic system. Trigeminal neurons innervate these tissues and can provide a direct connection to the CNS, brain, and/or spinal cord due, it is believed, to their role in the common chemical sense including mechanical sensation, thermal sensation and nociception (for example detection of hot spices and of noxious chemicals).

Delivery through the trigeminal neural pathway can employ lymphatics that travel with the trigeminal nerve to the pons and other brain areas and from there into dural lymphatics associated with portions of the CNS, such as the spinal cord. Transport along the trigeminal nerve can also deliver interferons to an olfactory bulb. A perivascular pathway and/or a hemangiolymphatic pathway, such as lymphatic channels running within the adventitia of cerebral blood vessels, can provide an additional mechanism for transport of therapeutic interferons to the spinal cord from tissue innervated by the trigeminal nerve.

The trigeminal nerve includes large diameter axons, which mediate mechanical sensation, *e.g.*, touch, and small diameter axons, which mediate pain and thermal sensation, both of whose cell bodies are located in the semilunar (or trigeminal) ganglion or the mesencephalic trigeminal nucleus in the midbrain. Certain portions of the trigeminal nerve extend into the nasal cavity, oral and conjunctival mucosa and/or epithelium. Other portions of the trigeminal nerve extend into the skin of the face, forehead, upper eyelid, lower eyelid, dorsum of the nose, side of the nose, upper lip, cheek, chin, scalp and teeth. Individual fibers of the trigeminal nerve collect into a large bundle, travel underneath the brain and enter the ventral aspect of the pons. Interferon can be administered to the trigeminal nerve, for example, through the nasal cavity's, oral, lingual, and/or conjunctival mucosa and/or epithelium; or through the skin of the face, forehead, upper eyelid, lower eyelid, dorsum of the nose, side of the nose, upper lip, cheek, chin, scalp and teeth. Such administration can employ extracellular or intracellular (*e.g.*, transneuronal) anterograde and retrograde transport of the interferon entering through the trigeminal nerves to the brain and its meninges, to the brain stem, or to the spinal cord. Once the interferon is dispensed into or onto tissue innervated by the trigeminal nerve, the interferon may transport through the tissue and travel along trigeminal neurons into areas of the CNS including the brain stem, cerebellum, spinal cord, olfactory bulb, and cortical and subcortical structures.

Delivery through the trigeminal neural pathway can employ movement of interferon across skin, mucosa, or epithelium into the trigeminal nerve or into a lymphatic, a blood vessel perivascular space, a blood vessel adventitia, or a blood vessel lymphatic that travels with the trigeminal nerve to the pons and from there into meningial lymphatics associated with portions of the CNS such as the spinal cord. Blood vessel lymphatics include lymphatic channels that are around the blood vessels on the outside of the blood vessels. This also is referred to as the hemangiolymphatic system. Introduction of an interferon into the blood vessel lymphatics does not necessarily introduce the interferon into the blood.

Neural Pathways and Nasal Administration

In one embodiment, the method of the invention can employ delivery by a neural pathway, *e.g.*, a trigeminal or olfactory neural pathway, after administration to the nasal cavity. Upon administration to the nasal cavity, delivery via the trigeminal neural pathway may employ movement of an interferon through the nasal mucosa and/or epithelium to reach a trigeminal nerve or a perivascular and/or lymphatic channel that travels with the nerve. Upon administration to the nasal cavity, delivery via the olfactory neural pathway may employ movement of an interferon through the nasal mucosa and/or epithelium to reach the olfactory nerve or a perivascular and/or lymphatic channel that travels with the nerve.

For example, the interferon can be administered to the nasal cavity in a manner that employs extracellular or intracellular (*e.g.*, transneuronal) anterograde and retrograde transport into and along the trigeminal and/or olfactory nerves to reach the brain, the brain stem, or the spinal cord. Once the interferon is dispensed into or onto nasal mucosa and/or epithelium innervated by the trigeminal and/or olfactory nerve, the interferon may transport through the nasal mucosa and/or epithelium and travel along trigeminal and/or olfactory neurons into areas of the CNS including the brain stem, cerebellum, spinal cord, olfactory bulb, and cortical and subcortical structures. Alternatively, administration to the nasal cavity can result in delivery of an interferon into a blood vessel perivascular space or a lymphatic that travels with the trigeminal and/or olfactory nerve to the pons, olfactory bulb, and other brain areas, and from there into meningeal lymphatics associated with portions of the CNS such as

the spinal cord. Transport along the trigeminal and/or olfactory nerve may also deliver interferons administered to the nasal cavity to the olfactory bulb, midbrain, diencephalon, medulla, and cerebellum. an interferon administered to the nasal cavity can enter the ventral dura of the brain and travel in lymphatic channels within the dura.

In addition, the method of the invention can be carried out in a way that employs a perivascular pathway and/or an hemangiolymphatic pathway, such as a lymphatic channel running within the adventitia of a cerebral blood vessel, to provide an additional mechanism for transport of interferon to the spinal cord from the nasal mucosa and/or epithelium. an interferon transported by the hemangiolymphatic pathway does not necessarily enter the circulation. Blood vessel lymphatics associated with the circle of Willis as well as blood vessels following the trigeminal and/or olfactory nerve can also be involved in the transport of the interferon.

Administration to the nasal cavity employing a neural pathway can deliver an interferon to the lymphatic system, brain stem, cerebellum, spinal cord, and cortical and subcortical structures. The interferon alone may facilitate this movement into the CNS, brain, and/or spinal cord. Alternatively, the carrier or other transfer-promoting factors may assist in the transport of the interferon into and along the trigeminal and/or olfactory neural pathway. Administration to the nasal cavity of a therapeutic interferon can bypass the blood-brain barrier through a transport system from the nasal mucosa and/or epithelium to the brain and spinal cord.

Neural Pathways and Transdermal Administration

In one embodiment, the method of the invention can employ delivery by a neural pathway, *e.g.*, a trigeminal neural pathway, after transdermal administration. Upon transdermal administration, delivery via the trigeminal neural pathway may employ movement of an interferon through the skin to reach a trigeminal nerve or a perivascular and/or lymphatic channel that travels with the nerve.

For example, the interferon can be administered transdermally in a manner that employs extracellular or intracellular (*e.g.*, transneuronal) anterograde and retrograde transport into and along the trigeminal nerves to reach the brain, the brain stem, or the spinal cord. Once the interferon is dispensed into or onto skin innervated

by the trigeminal nerve, the interferon may transport through the skin and travel along trigeminal neurons into areas of the CNS including the brain stem, cerebellum, spinal cord, olfactory bulb, and cortical and subcortical structures. Alternatively, transdermal administration can result in delivery of an interferon into a blood vessel perivascular space or a lymphatic that travels with the trigeminal nerve to the pons, olfactory bulb, and other brain areas, and from there into meningeal lymphatics associated with portions of the CNS such as the spinal cord. Transport along the trigeminal nerve may also deliver transdermally administered interferons to the olfactory bulb, midbrain, diencephalon, medulla and cerebellum. The ethmoidal branch of the trigeminal nerve enters the cribriform region. An transdermally administered interferon can enter the ventral dura of the brain and travel in lymphatic channels within the dura.

In addition, the method of the invention can be carried out in a way that employs a perivascular pathway and/or an hemangiolymphatic pathway, such as a lymphatic channel running within the adventitia of a cerebral blood vessel, to provide an additional mechanism for transport of interferon to the spinal cord from the skin. an interferon transported by the hemangiolymphatic pathway does not necessarily enter the circulation. Blood vessel lymphatics associated with the circle of Willis as well as blood vessels following the trigeminal nerve can also be involved in the transport of the interferon.

Transdermal administration employing a neural pathway can deliver an interferon to the brain stem, cerebellum, spinal cord and cortical and subcortical structures. The interferon alone may facilitate this movement into the CNS, brain, and/or spinal cord. Alternatively, the carrier or other transfer-promoting factors may assist in the transport of the interferon into and along the trigeminal neural pathway. Transdermal administration of a therapeutic interferon can bypass the blood-brain barrier through a transport system from the skin to the brain and spinal cord.

Neural Pathways and Sublingual Administration

In another embodiment, the method of the invention can employ delivery by a neural pathway, *e.g.*, a trigeminal neural pathway, after sublingual administration. Upon sublingual administration, delivery via the trigeminal neural pathway may

employ movement of an interferon from under the tongue and across the lingual epithelium to reach a trigeminal nerve or a perivascular or lymphatic channel that travels with the nerve.

For example, the interferon can be administered sublingually in a manner that employs extracellular or intracellular (*e.g.*, transneuronal) anterograde and retrograde transport through the oral mucosa and then into and along the trigeminal nerves to reach the brain, the brain stem, or the spinal cord. Once the interferon is administered sublingually, the interferon may transport through the oral mucosa by means of the peripheral processes of trigeminal neurons into areas of the CNS including the brain stem, spinal cord and cortical and subcortical structures. Alternatively, sublingual administration can result in delivery of an interferon into lymphatics that travel with the trigeminal nerve to the pons and other brain areas and from there into meningeal lymphatics associated with portions of the CNS such as the spinal cord. Transport along the trigeminal nerve may also deliver sublingually administered interferons to the olfactory bulbs, midbrain, diencephalon, medulla and cerebellum. The ethmoidal branch of the trigeminal nerve enters the cribriform region. A sublingually administered interferon can enter the ventral dura of the brain and travel in lymphatic channels within the dura.

In addition, the method of the invention can be carried out in a way that employs an hemangiolymphatic pathway, such as a lymphatic channel running within the adventitia of a cerebral blood vessel, to provide an additional mechanism for transport of an interferon to the spinal cord from the oral submucosa. An interferon transported by the hemangiolymphatic pathway does not necessarily enter the circulation. Blood vessel lymphatics associated with the circle of Willis as well as blood vessels following the trigeminal nerve can also be involved in the transport of the interferon.

Sublingual administration employing a neural pathway can deliver an interferon to the brain stem, cerebellum, spinal cord and cortical and subcortical structures. The interferon alone may facilitate this movement into the CNS, brain, and/or spinal cord. Alternatively, the carrier or other transfer-promoting factors may assist in the transport of the interferon into and along the trigeminal neural pathway.

Sublingual administration of a therapeutic interferon can bypass the blood-brain barrier through a transport system from the oral mucosa to the brain and spinal cord.

Neural Pathways and Conjunctival Administration

In another embodiment, the method of the invention can employ delivery by a neural pathway, *e.g.* a trigeminal neural pathway, after conjunctival administration. Upon conjunctival administration, delivery via the trigeminal neural pathway may employ movement of an interferon from the conjunctiva through the conjunctival epithelium to reach the trigeminal nerves or lymphatic channels that travel with the nerve.

For example, the interferon can be administered conjunctivally in a manner that employs extracellular or intracellular (*e.g.*, transneuronal) anterograde and retrograde transport through the conjunctival mucosa and then into and along the trigeminal nerves to reach the brain, the brain stem, or the spinal cord. Once the interferon is administered conjunctivally, the interferon may transport through the conjunctival mucosa by means of the peripheral processes of trigeminal neurons into areas of the CNS including the brain stem, spinal cord and cortical and subcortical structures. Alternatively, conjunctival administration can result in delivery of an interferon into lymphatics that travel with the trigeminal nerve to the pons and other brain areas and from there into meningeal lymphatics associated with portions of the CNS such as the spinal cord. Transport along the trigeminal nerve may also deliver conjunctivally administered interferons to the olfactory bulbs, midbrain, diencephalon, medulla and cerebellum. The ethmoidal branch of the trigeminal nerve enters the cribriform region. An conjunctivally administered interferon can enter the ventral dura of the brain and travel in lymphatic channels within the dura.

In addition, the method of the invention can be carried out in a way that employs an hemangiolymphatic pathway, such as a lymphatic channel running within the adventitia of cerebral blood vessel, to provide an additional mechanism for transport of an interferon to the spinal cord from the conjunctival submucosa. An interferon transported by the hemangiolymphatic pathway does not necessarily enter the circulation. Blood vessel lymphatics associated with the circle of Willis as well as

blood vessels following the trigeminal nerve can also be involved in the transport of the interferon.

Conjunctival administration employing a neural pathway can deliver an interferon to the brain stem, cerebellum, spinal cord and cortical and subcortical structures. The interferon alone may facilitate this movement into the CNS, brain, and/or spinal cord. Alternatively, the carrier or other transfer-promoting factors may assist in the transport of the interferon into and along the trigeminal neural pathway. Conjunctival administration of a therapeutic interferon can bypass the blood-brain barrier through a transport system from the conjunctival mucosa to the brain and spinal cord.

Pharmaceutical Composition: Anti-CD40 Antibodies

The anti-CD40 antibodies of the invention are administered at a concentration that is therapeutically effective to prevent or treat B cell or other antigen presenting cell mediated diseases such as multiple sclerosis in a patient also receiving interferon therapy as discussed herein. To accomplish this goal, the anti-CD40 antibodies may be formulated using a variety of acceptable excipients known in the art. Typically, the anti-CD40 antibodies are administered by injection, either intravenously or parenterally. Methods to accomplish this administration are known to those of ordinary skill in the art. Compositions comprising anti-CD40 antibodies which may be topically or orally administered, or which may be subcutaneously administered, or which may be capable of transmission across mucous membranes, are also suitable.

Before administration to patients, formulants may be added to the antibodies. A liquid formulation is preferred. For example, these formulants may include oils, polymers, vitamins, carbohydrates, amine acids, salts, buffers, albumin, surfactants, or bulking agents. Preferably carbohydrates include sugar or sugar alcohols such as mono-, di-, or polysaccharides, or water soluble glucans. The saccharides or glucans can include fructose, dextrose, lactose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch, hydroxethyl starch and carboxymethylcellulose, or mixtures thereof. Sucrose is most preferred. "Sugar alcohol" is defined as a C_4 to C_8 hydrocarbon having an --OH group and includes galactitol, inositol, mannitol, xylitol, sorbitol, glycerol, and

arabitol. Mannitol is most preferred. These sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. Preferably, the sugar or sugar alcohol concentration is between 1.0 w/v % and 7.0 w/v %, more preferable between 2.0 and 6.0 w/v %. Preferably amino acids include levorotary (L) forms of carnitine, arginine, and betaine; however, other amino acids may be added. Preferred polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000. It is also preferred to use a buffer in the composition to minimize pH changes in the solution before lyophilization or after reconstitution. Most any physiological buffer may be used, but titrate, phosphate, succinate, and glutamate buffers or mixtures thereof are preferred. Most preferred is a citrate buffer. Preferably, the concentration is from 0.01 to 0.3 molar. Surfactants that can be added to the formulation are shown in EP Nos. 270,799 and 268,110.

Additionally, antibodies can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Preferred polymers are polyoxyethylene polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: $R(O--CH_2--CH_2)_n O--R$ where R can be hydrogen, or a protective group such as an alkyl or alkanol group. Preferably, the protective group has between 1 and 8 carbons, more preferably it is methyl. The symbol n is a positive integer, preferably between 1 and 1,000, more preferably between 2 and 500. The PEG has a preferred average molecular weight between 1000 and 40,000, more preferably between 2000 and 20,000, most preferably between 3,000 and 12,000. Preferably, PEG has at least one hydroxy group, more preferably it is a terminal hydroxy group. It is this hydroxy group which is preferably activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/antibody of the present invention. Preferred polymers, and methods to attach them to peptides, are shown in U.S. Pat. Nos. 4,766,106; 4,179,337; 4,495,285; and 4,609,546 which are all hereby incorporated by reference in their entireties.

Water soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), etc. POG is preferred, in part because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di-, triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body. The POG has a preferred molecular weight in the same range as PEG. The structure for POG is shown in Knauf et al., *J. Bio. Chem. 263*:15064-15070, 1988, and a discussion of POG/IL-2 conjugates is found in U.S. Pat. No. 4,766,106, both of which are hereby incorporated by reference in their entireties.

Another drug delivery system for increasing circulatory half-life is the liposome. Methods of preparing liposome delivery systems are known in the art, *see, e.g.*, Gabizon et al., *Cancer Research 42*:4734, 1982; Cafiso, *Biochem Biophys Acta 649*:129, 1981; and Szoka, *Ann Rev Biophys Eng 9*:467, 1980. Other drug delivery systems are known in the art, *see, e.g.*, Poznansky et al., Drug Delivery Systems (R. L. Juliano, ed., Oxford, N.Y. 1980), pp. 253-315; M. L. Poznansky, *Pharm Revs 36*:277, 1984.

After the liquid pharmaceutical composition is prepared, it is preferably lyophilized to prevent degradation and to preserve sterility. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior to use, the composition may be reconstituted with a sterile diluent (Ringer's solution, distilled water, or sterile saline, for example) which may include additional ingredients. Upon reconstitution, the composition is preferably administered to subjects using those methods that are known to those skilled in the art.

The preferred route of administration is parenterally, but subcutaneous and intramuscular administration are also suitable. In parenteral administration, the compositions of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic and nontherapeutic. Examples of such vehicles are saline, Ringer's solution, dextrose solution, and Hanks' solution. Nonaqueous vehicles such as fixed oils and ethyloleate may also be used. A preferred vehicle is 5% dextrose in saline. The vehicle may

contain minor mounts of additives such as substances that enhance isotonicity and chemical stability, including buffers and preservatives.

The dosage and mode of administration will depend on the individual. Generally, the compositions are administered so that antibodies are given at a dose between 1 μg/kg and 20 mg/kg, more preferably between 20 μg/kg and 10 mg/kg, most preferably between 1 and 7 mg/kg. Suitably, it is given as an infusion or as a bolus dose, to increase circulating levels by 10-20 fold and for 4-6 hours after the bolus dose. Continuous infusion may also be used after the bolus dose. If so, the antibodies may be infused at a dose between 5 and 20 μg/kg/minute, more preferably between 7 and 15 μg/kg/minute. Suitable treatment regimens are disclosed in WO 00/27428 and WO 00/27433, which are incorporated herein by reference.

Additional suitable formulations and routes of administration are discussed below and in U.S. Patent No. 5,874,082, incorporated herein by reference. Typically, the antibodies are administered by injection, either intravenously or parenterally. It may also be possible to obtain compositions which may be topically or orally administered, or which may be capable of transmission across mucous membranes. Compositions comprising antibodies may be administered subcutaneously.

Before administration to patients, formulants may be added to the antibodies. A liquid formulation is preferred. For example, these formulants may include oils, polymers, vitamins, carbohydrates, amino acids, salts, buffers, albumin, surfactants, or bulking agents. Preferably carbohydrates include sugar or sugar alcohols such as mono-, di- or polysaccharides, or water soluble glucans. The saccharide or glucans can include fructose, dextrose, lactose, glucose, mannose, sorbose, xylose, maltose, sucrose, dextran, pullulan, dextrin, alpha and beta cyclodextrin, soluble starch, hydroxethyl starch and carboxymethylellulose, or mixtures thereof. Sucrose is most preferred. "Sugar alcohol" is defined as a C_4 to C_8 hydrocarbon having an –OH group and includes galacitol, inositol, mannitol, xylitol, sorbitol, glycerol, and arabitol. Mannitol is most preferred. These sugars or sugar alcohols mentioned above may be used individually or in combination. There is no fixed limit to amount used as long as the sugar or sugar alcohol is soluble in the aqueous preparation. Preferably, the sugar or sugar alcohol concentration is between 1.0 w/v % and 7.0 w/v %, more preferable between 2.0 and 6.0 w/v %. Preferably amino acids include levorotary (L) forms of

canitine, arginine, and betaine; however, other amino acids may be added. Preferred polymers include polyvinylpyrrolidone (PVP) with an average molecular weight between 2,000 and 3,000, or polyethylene glycol (PEG) with an average molecular weight between 3,000 and 5,000. It is also preferred to use a buffer in the composition to minimize pH changes in the solution before lyophilization or after reconstitution. Most any physiological buffer may be used, but citrate, phosphate, succinate, and glutamate buffers or mixtures thereof are preferred. Most preferred is a citrate buffer. Preferably, the concentration is from 0.01 to 0.3 molar. Surfactants that can be added to the formulation are shown in EP Nos. 270,799 and 268,110.

Additionally, antibodies can be chemically modified by covalent conjugation to a polymer to increase their circulating half-life, for example. Preferred polymers are disclosed in U.S. Patent Nos. 4,179,337; 4,495,285; and 4,609,546 which are all hereby incorporated by reference in their entireties. Preferred polymers are polyoxyethylated polyols and polyethylene glycol (PEG). PEG is soluble in water at room temperature and has the general formula: $R(O—CH_2—CH_2)_nO—R$ where R can be hydrogen, or a protective group such as an alkyl or aklanol group. Preferably, the protective group has between 1 and 8 carbons, more preferably it is methyl. The symbol n is a positive integer, preferably between 1 and 1,000, more preferably between 2 and 500. The PEG has a preferred average molecular weight between 1,000 and 40,000, more preferably between 2,000 and 20,000, most preferably between 3,000 and 12,000. Preferably, PEG has at least one hydroxy group, more preferably it is a terminal hydroxy group. It is this hydroxy group which is preferably activated to react with a free amino group on the inhibitor. However, it will be understood that the type and amount of the reactive groups may be varied to achieve a covalently conjugated PEG/antibody of the present invention.

Water soluble polyoxyethylated polyols are also useful in the present invention. They include polyoxyethylated sorbitol, polyoxyethylated glucose, polyoxyethylated glycerol (POG), etc. POG is preferred, in part because the glycerol backbone of polyoxyethylated glycerol is the same backbone occurring naturally in, for example, animals and humans in mono-, di- and triglycerides. Therefore, this branching would not necessarily be seen as a foreign agent in the body. The POG has a preferred molecular weight in the same range as PEG. The structure for POG is

shown in Knauf et al., *J. Bio. Chem. 263*:15064-15070, 1988, and a discussion of POG/IL-2 conjugates is found in U.S. Pat. No. 4,766,106, both of which are hereby incorporated by reference in their entireties.

Another drug delivery system for increasing circulatory half-life is the liposome. Methods of preparing liposome delivery systems are discussed in Gabizon et al., *Cancer Research, 42*:4734, 1982; Cafiso, *Biochem. Biophys. Acta 649*:129, 1981; and Szoka, *Ann. Rev. Biophys. Eng. 9*:467, 1980. Other drug delivery systems are known in the art and are described in, *e.g.*, Poznansky et al., DRUG DELIVERY SYSTEMS (R.L. Juliano, Ed., Oxford, N.Y. 1980), pp. 253-315; M.L. Poznansky, *Pharm. Revs. 36*:277, 1984.

After the liquid pharmaceutical composition is prepared, it is preferably lyophilized to prevent degradation and to preserve sterility. Methods for lyophilizing liquid compositions are known to those of ordinary skill in the art. Just prior to use, the composition may be reconstituted with a sterile diluent (Ringer's solution, distilled water, or sterile saline, for example) which may include additional ingredients. Upon reconstitution, the composition is preferably administered to subjects using those methods that are known to those skilled in the art.

One preferred route of administration is parenterally. In parenteral administration, the compositions of this invention will be formulated in a unit dosage injectable form such as a solution, suspension or emulsion, in association with a pharmaceutically acceptable parenteral vehicle. Such vehicles are inherently nontoxic and nontherapeutic. Examples of such vehicles are saline, Ringer's solution, dextrose solution, and Hanks' solution. Nonaqueous vehicles such as fixed oils and ethyl oleate may also be used. A preferred vehicle is 5% dextrose in saline. The vehicle may contain minor amounts of additives such as substances that enhance isotonicity and chemical stability, including buffers and preservatives.

The dosage and mode of administration will depend on the individual. Generally, the compositions are administered so that antibodies are given at a dose between 1 μg/kg and 20 mg/kg, more preferably between 20 μg/kg and 10 mg/kg, most preferably between 0.3 and 7 mg/kg. Preferably, it is given as a bolus dose, to increase circulating levels by 10-20 fold and for 4-6 hours after the bolus dose. Continuous infusion may also be used after the bolus dose. If so, the antibodies may

be infused at a dose between 5 and 20 μg/kg/minute, more preferably between 7 and 15 μg/kg/minute.

The anti-CD40 antibodies suitable for use in the invention include those disclosed in U.S. Patent Nos. 5,874,082; 6,004,552; 6,056,959; 5,677,165; and 6,051,228, all of which are incorporated herein by reference.

Articles and Methods of Manufacture

The present invention also includes an article of manufacture providing an interferon and an anti-CD40 antibody for administration to the CNS, brain, and/or spinal cord. The article of manufacture can include one or more vials or other containers that contain a composition suitable for the present method together with any carrier, either dried or in liquid form. The interferon and anti-CD40 antibody will preferably be supplied in separate vials. However, they may be co-administered, or administered within minutes, hours, or days of each other. The article of manufacture further includes instructions in the form of a label on the container and/or in the form of an insert included in a box in which the container is packaged, for the carrying out the method of the invention. The instructions can also be printed on the box in which the vial is packaged. The instructions contain information such as sufficient dosage and administration information so as to allow the subject or a worker in the field to administer the interferon and an anti-CD40 antibody. It is anticipated that a worker in the field encompasses any doctor, nurse, technician, spouse, or other care-giver. The interferon and an anti-CD40 antibody can also be self-administered by the subject.

According to the invention, the interferon and an anti-CD40 antibody can be used for manufacturing a composition or medicament suitable for intranasal, conjunctival, transdermal, and/or sublingual administration. For example, a liquid or solid composition can be manufactured in several ways, using conventional techniques. A liquid composition can be manufactured by dissolving an interferon in a suitable solvent, such as water, at an appropriate pH, including buffers or other excipients, for example to form a solution described herein above.

IFN-β, like many of the interferons, reportedly serves as an immunomodulator on a number of target cells (Hall et al., *J. Neuroimmunol.* 72:11-19, 1997). For instance, IFN-β appears to exert antiproliferative action on macrophages, counteract

"the mitogenic stimulus of certain interferons", augment natural killer cell activity to induce an increase in the production of cytotoxic T lymphocytes, and act on large, granular lymphocytes to increase killer cell activity. Additionally, IFN-β augments the proliferation of B cells and the secretion of IgM, IgG, and IgA. It has been shown to upregulate class I MHC expression to produce an increase in the presentation of class I restricted antigen CD8 cells (Hall et al., *J. Neuroimmunol. 72*:11-19, 1997). Conversely, IFNβ exerts an inhibitory effect on the upregulation of class II surface expression. Hence, the immunomodulatory activities of IFN-β include, for example, influencing systemic immune function, antigen presentation, interferon production, and entry of leukocytes into the CNS (Yong et al., *Neurology 51*:582-689, 1998). Direct delivery of the interferon to the lymphatics of the head and neck using the administration methods of the present invention allows the interferon to modulate the immune response, *i.e.*, influence chronic and acute inflammation, wound healing, and the autoimmune response; modulate the function by lymphocytes (reduce lymphocyte infiltration of the injured tissue); etc.

Given the immunomodulatory role of interferons, the present invention can be employed to deliver interferons, preferably IFN-β, and anti-CD40 antibodies to various tissues of the head and neck for the treatment and/or prevention of diseases or disorders characterized by immune and inflammatory responses. The disorder or disease of particular interest is multiple sclerosis.

MS presents in the white matter of the CNS and spinal cord as a number of sclerotic lesions or plaques (Prineas, *Demyelinating Diseases*, Elsvevier: Amsterdam 1985; Raine, *Multiple Sclerosis*, Williams and Wilkins: Baltimore, 1983; Raine et al., *J. Neuroimmunol. 20*:189-201, 1988; and Martin, *J. Neural Transmission (Suppl) 49*:53-67, 1997). The characteristic MS lesion is inflamed, exhibits axonal demyelination, axonal degeneration, and is found around small venules. These characteristics typically evolve early in plaque development and are hypothesized to occur as a result of a breakdown in the blood-brain barrier (BBB). As a consequence of BBB breakdown, infiltrates consisting of various lymphocytes and macrophages enter the brain. The infiltrates cause a decrease in inflammation while increasing the presence of glial scar tissue, and elicit incomplete remyelination (Martin, *J. Neural Transmission (Suppl) 49*:53-67, 1997). Further, it is hypothesized that this apparent

immunologic attack targets not only the myelin sheath, but also the oligodendrocytes imperative to CNS myelin production. Interferons are known to effectively reduce the symptoms of MS. For example, interferon-β (IFN-β) has received interest as a treatment for relapsing-remitting MS. In addition, interest has also developed in the use of interferon-τ as an effective treatment in autoimmune diseases, such as MS. *See*, for example, U.S. Patent No. 6,060,450, herein incorporated by reference.

The immunomodulating activity of IFN-β influences the clinical symptoms of MS. While the present invention is not bound by the mechanism of IFN-β action, the central nervous system damage that ensues in MS patients is believed to be due to the delayed-type hypersensitivity response. This is a cell-mediated response. First, T cells are activated by antigens and conveyed to the lymphoid organ (activation). The lymphoid organ then activates these T cells while continuing to recruit more T cells to its site (recruitment). The activated lymphocytes proliferate and return to circulation (expansion). Once returned to circulation, the activated lymphocytes migrate through the blood stream, crossing endothelial cells lining the capillaries (migration). These migrating lymphocytes and macrophages target, and are attracted to the area of inflammation (attraction). Resulting from this attraction, other lymphocytes continue to the area of inflammation and tissue is destroyed (tissue destruction). Subsequently, the acute response is suppressed (via tissue destruction), and repair of the area of inflammation, which is quite limited in MS, may commence (repair) (Kelley, *J. of Neuroscience Nursing 28*:114-120, 1996). Therefore, the migration of activated lymphocytes from the blood initiates the immune response, thereby allowing BBB penetration of activated lymphocytes.

Evidence suggests that the immunomodulatory activity of IFN-β inhibits IFN-γ upregulation by inhibiting the expansion stage of the delayed-type hypersensitivity response and thereby influences the clinical symptoms of MS. Particularly, the reduction of myelin damage appears to occur as a result of two hypothesized mechanisms of IFN-β action: (1) inhibition of IFN-γ-induced macrophage activation, and (2) inhibition of monocytotic TNF release (Kelly, *J. Neuroscience Nursing 28*:114-120, 1996). Potential sites of IFN-β action construed by these hypotheses involve systemic immune function, antigen presentation, interferon production, and entry of

leukocytes into the CNS (Yong et al., *Neurology 51*:682-689, 1998). Each of these sites has been elaborated in human and animal experiments of MS.

An "effective amount" of an interferon is an amount sufficient to prevent, treat, reduce and/or ameliorate the symptoms and/or underlying causes of MS. In some instances, an "effective amount" is sufficient to eliminate the symptoms of those diseases and, perhaps, overcome the disease itself. An important aspect of the invention is that the dose of interferon may be decreased with the administration of the anti-CD40 antibody. In addition, interferon treatment may be continued for a longer time if the patient is also receiving anti-CD40 antibody therapy. In the context of the present invention, the terms "treat" and "therapy" and the like refer to alleviate, slow the progression, prophylaxis, attenuation or cure of existing disease. Prevent, as used herein, refers to delaying, slowing, inhibiting, reducing or ameliorating the onset of MS. It is preferred that a sufficient quantity of the interferon be applied in non-toxic levels in order to provide an effective level of activity within the CNS to prevent or treat MS. The methods of the present invention may be used with any mammal. Exemplary mammals include, but are not limited to rats, cats, dogs, horses, cows, sheep, pigs, and more preferably humans.

An effective amount of an interferon to treat MS using the administration methods of the present invention will be sufficient to reduce or lessen the clinical symptoms of MS. For instance, experimental allergic encephalomyelitis (EAE) is commonly used as an animal model of MS. A therapeutically effect amount of an interferon delivered by the methods of the present invention will be such as to improve the clinical symptoms of EAE in the experimental animal (*i.e.*, rats or mice). EAE in rats is scored on a scale of 0-4: 0, clinically normal; 1, flaccid tail paralysis; 2, hind limb weakness; 3, hind limb paralysis; 4, front and hind limb affected. An effective amount of interferon delivered by the methods of the present invention will be effective if there is at least a 30%, 40%, 50% or greater reduction in the mean cumulative score over several days following the onset of disease symptoms in comparison to the control group.

Furthermore, effective treatment of MS may be examined in several alternative ways including extended disability status scale (EDSS) , appearance of exacerbations, or MRI. If any of these indicia show that the interferon treatment is losing its

effectiveness, or if symptoms worsen, then co-treatment with anti-CD40 antibodies is warranted. According to the invention, the anti-CD40 antibody treatment will allow the continued administration of interferon. Satisfying any of the following criteria evidences effective treatment.

The EDSS is a means to grade clinical impairment due to MS (Kurtzke, *Neurology 33*:1444, 1983). Eight functional systems are evaluated for the type and severity of neurologic impairment. Briefly, prior to treatment, impairment in the following systems is evaluated: pyramidal, cerebellar, brainstem, sensory, bowel and bladder, visual, cerebral, and other. Follow-ups are conducted at defined intervals. The scale ranges from 0 (normal) to 10 (death due to MS). A decrease of one full step defines an effective treatment in the context of the present invention (Kurtzke, *Ann. Neurol. 36*:573-579, 1994); a decrease of 0.5 step if EDSS score is >5.5 is also within the definition of effective treatment.

Exacerbations are defined as the appearance of a new symptom that is attributable to MS and accompanied by an appropriate new neurologic abnormality (IFN-β MS Study Group, *supra*). In addition, the exacerbation must last at least 24 hours and be preceded by stability or improvement for at least 30 days. Standard neurological examinations result in the exacerbations being classified as either mild, moderate, or severe according to changes in a Neurological Rating Scale (Sipe et al., *Neurology 34*:1368, 1984). An annual exacerbation rate and proportion of exacerbation-free patients are determined. Therapy is deemed to be effective if there is a statistically significant difference in the rate or proportion of exacerbation-free patients between the treated group and the placebo group for either of these measurements. In addition, time to first exacerbation and exacerbation duration and severity may also be measured. A measure of effectiveness as therapy in this regard is a statistically significant difference in the time to first exacerbation or duration and severity in the treated group compared to control group.

MRI can be used to measure active lesions using gadolinium-DTPA-enhanced imaging (McDonald et al., *Ann. Neurol. 36*:14, 1994) or the location and extent of lesions using T_1 and T_2 -weighted techniques. Briefly, baseline MRIs are obtained. The same imaging plane and patient position are used for each subsequent study. Areas of lesions are outlined and summed slice by slice for total lesion area. Three

analyses may be done: evidence of new lesions, rate of appearance of active lesions, and percentage change in lesion area (Paty et al., *Neurology 43*:665, 1993). Improvement due to therapy is established when there is a statistically significant improvement in an individual patient compared to baseline or in a treated group versus a placebo group.

The present invention will now be illustrated by reference to the following examples which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are illustrative and are not to be construed as restricting the invention in any way.

EXAMPLES

Patient populations are selected on the basis of the following criteria: symptoms of MS that are incompletely alleviated by administration of interferon-β1b or other MS therapeutics, including indicia for discontinuing interferon-β1b treatment because of decreased effectiveness. These criteria are evaluated using standard methods of measuring MS disease activity and progression. Preferred criteria for selecting patients are (a) at least one relapse in the previous six months despite treatment with interferon beta-1b or other MS therapeutics; (b) at least one enhancing lesion at baseline MRI scan; clinically significant progression in disability over the previous six months despite treatment with interferon beta-1b or other MS therapeutics. The patients are divided into two treatment groups and given a treatment option as described in Examples 1 and 2.

EXAMPLE 1

TREATMENT WITH ANTI-CD40 ANTIBODY

Patients will be evaluated at baseline for EDSS score, number of enhancing lesions on MRI scan, and lesion volume on MRI scan. These measures will be obtained on a repeated basis over the course of 1-2 years. Patients will receive an anti-CD40 antibody such as 15B8 in a single cycle at a dose of 0.03 mg/kg to 10 mg/kg via intravenous infusion weekly for four to eight doses. Efficacy of the anti-CD40 antibody in reducing the frequency of enhancing lesions and lesion volume on MRI and reduction in the proportion of patients experiencing a confirmed disease

progression will be assessed at yearly intervals. Clinical and MRI course of patients receiving the anti-CD40 antibody will be compared against patients receiving a matching placebo.

EXAMPLE 2

TREATMENT WITH ANTI-CD40 ANTIBODY AND β-INTERFERON

Patients will be evaluated at baseline for EDSS score, number of enhancing lesions on MRI scan, and lesion volume on MRI scan. These measures will be obtained on a repeated basis over the course of 1-2 years. Some patients will receive an anti-CD40 antibody such as 15B8 in a single cycle at a dose of 0.03 mg/kg to 10 mg/kg via intravenous infusion weekly for four to eight doses. Some patients will receive a matching placebo on the same time course as those receiving the anti-CD40 antibody. All patients will also receive the commercially approved dose of beta-interferon (8 MIU subcutaneously every other day for interferon beta-1b [Betaseron®], 6 MIU every other day intramuscularly for interferon beta-1a [Avonex®] or 12 MIU every other day subcutaneously for interferon beta-1a [Rebif® if approved for use in the US at the time of this study]. Efficacy of the anti-CD40 antibody in reducing the frequency of enhancing lesions and lesion volume on MRI and reduction in the proportion of patients experiencing a confirmed disease progression will be assessed at yearly intervals. Clinical and MRI course of patients receiving the anti-CD40 antibody plus interferon will be compared against patients receiving interferon alone.

EXAMPLE 3

EVALUATION OF PATIENT TREATMENTS

Patients treated as described in Examples 1 and 2 are evaluated to determine disease progression. In one test, the brain lesions are evaluated by MRI.

The first set of experiments is a proof of concept study to show benefit of anti-CD40 antibody either alone in patients who are refractory to other therapies including interferon or in combination with continued use of Interferon-β-1b in patients at high risk for discontinuation of therapy (MRI based primary endpoint with clinical secondary endpoints). A second Phase III pivotal study of 15B8 as monotherapy

versus add-on therapy following failed treatment with interferon or glatirimer acetate (clinical endpoint based primary endpoint with MRI based secondary endpoints) is then conducted.

The present invention has been described with reference to specific embodiments. However, this application is intended to cover those changes and substitutions which may be made by those skilled in the art without departing from the spirit and the scope of the appended claims.

What is claimed:

1. A method of treating multiple sclerosis comprising contacting B cells of a patient in need of treatment with an anti-CD40 antibody, wherein said anti-CD40 antibody inhibits B cell differentiation or B cell proliferation.

2. The method of claim 1, wherein a type 1 interferon, or biologically active fragment thereof, is co-administered to said patient in need of treatment.

3. The method of claim 2,wherein the type 1 interferon is beta-interferon.

4. The method of claim 2, wherein said interferon or fragment thereof, is a variant interferon.

5. The method of claim 2, wherein said patient has relapsing and remitting multiple sclerosis.

6. The method of claim 4, wherein said interferon is administered intranasally.

7. The method of claim 4, wherein the dose of said interferon is between 0.14 nmol/kg to 138 nmol/kg.

8. The method according to claims 4, 5, 6, or 7, wherein said dose of interferon is administered intermittently.

9. The method of claim 8, wherein said dose of interferon is administered in a cyclic regiment.

10. The method according to claim 1, 2, 3, 4, 5, 6, 7, 8 or 9, wherein said anti-CD40 antibody is administered at a dose between 1ug/kg and 20ug/kg.

11. A method of treating Crohn's disease comprising contacting B cells of a patient in need of treatment with an anti-CD40 antibody, wherein said anti-CD40 antibody inhibits B cell differentiation or B cell proliferation.

12. The method of claim 11, wherein a type 1 interferon, or biologically active fragment thereof, is co-administered to said patient in need of treatment.

13. The method of claim 12,wherein the type 1 interferon is beta-interferon.

14. The method according to claims 12 or 13, wherein said interferon or fragment thereof, is a variant interferon.0.14 nmol/kg to 138 nmol/kg.

15. The method according to claim 14, wherein the dose of said interferon is between 0.14 nmol/kg to 138 nmol/kg.

16. The method according to claims 11, 12, 13, 14, or 15, wherein said anti-CD40 antibody is administered at a dose between 1ug/kg and 20ug/kg.

SEQUENCE LISTING

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Kwan, Min Fun

<120> ANTAGONIST ANTI-CD40 MONOCLONAL ANTIBODY THERAPY FOR MULTIPLE SCLEROSIS TREATMENT

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